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Climate-dependent variation in cold tolerance of weedy rice and rice mediated by *OsICE1* promoter methylation

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Abstract

The mechanisms by which weedy rice (*Oryza sativa* f. *spontanea*) has adapted to endure low-temperature stress in northern latitudes remain unresolved. In this study, we assessed cold tolerance of 100 rice varieties and 100 co-occurring weedy rice populations, which were sampled across a broad range of climates in China. A parallel pattern of latitude-dependent variation in cold tolerance was detected in cultivated rice and weedy rice. At the molecular level, differential cold tolerance was strongly correlated with relative expression levels of *CBF* cold response pathway genes and with methylation levels in the promoter region of *OsICE1*, a regulator of this pathway. Among all methylated cytosine sites of the *OsICE1* promoter, levels of CHG and CHH methylation were found to be significantly correlated with cold tolerance among accessions. Furthermore, within many of the collection locales, weedy rice shared identical or near-identical *OsICE1* methylation patterns with co-occurring cultivated rice. These findings provide new insights on the possible roles that methylation variation in the *OsICE1* promoter may play in cold tolerance, and they suggest that weedy rice can rapidly acquire cold tolerance via methylation patterns that are shared with co-occurring rice cultivars.

KEYWORDS

CBF pathway, climatic adaptation, cold tolerance, DNA methylation, OsICE1, weedy rice

1 | INTRODUCTION

Cold stress is a major environmental factor that limits the geographical distributions of plant species and has serious impacts on the growth and development of plants adapted to warm climates (Boyer, 1982). Rice (*Oryza sativa* L.) originated in tropical or subtropical areas and is a naturally warm-adapted species that is sensitive to low temperatures; the optimum temperature for rice growth is 15–35°C. Temperatures below 15°C retard rice growth and development and can cause serious physiological damage (Andaya & Tai, 2006; Fujino et al., 2004). Currently, the area of rice cultivation in China is gradually expanding from the south into northern temperate regions, where varieties that are adapted to warmer climates may suffer from low-temperature stress, affecting crop establishment and reproductive stages (Wang et al., 2014). Low-temperature stress causes 3–5 million tonnes of rice yield losses in China every year (Zhang, Jiang, & Wang, 2013), and rice grown in cool temperate regions must have a requisite level of cold tolerance to be productive in these climates.

Concomitant with the spread of rice cultivation into northern climates, weedy rice (*Oryza sativa* L.) has rapidly adapted to the same cool climates. Weedy rice is a major agricultural weed of rice production fields worldwide that is morphologically and physiologically similar to rice, threatening the yield and quality of rice harvests

Xie, Han and Li contributed equally to the manuscript.

(Delouche, Burgos, & Gealy, 2007; Qiu et al., 2017; Ziska et al., 2015). Substantial evidence indicates that many weedy rice ecotypes are directly descended from cultivated rice (especially from early landraces that predate modern cultivars) and are closely related to the crop (He, Kim, & Park, 2017; Ishikawa et al., 2005; Kane & Baack, 2007; Li, Li, Jia, Caicedo, & Olsen, 2017; Qiu et al., 2017, 2014; Reagon et al., 2010; Song, Chuah, Tam, & Olsen, 2014; Sun et al., 2013; Zhang et al., 2015, 2018). Almost all rice cultivation areas globally are affected by weedy rice, which suggests that this weed can adapt quickly to a range of different local environmental conditions. In the case of cold adaptation, the temperature required for weedy rice seed germination has been found to be lower for populations originating from high latitudes than those from lower latitudes. This allows weedy rice seeds to avoid growing in inappropriate environments via changes in critical temperature cues for seed germination (Xia, Xia, Ellstrand, Yang, & Lu, 2011). While both cultivated and weedy rice are primarily self-fertilizing, outcrossing can occur, and genetic introgression from local cultivated rice into co-occurring weed populations could potentially promote the weed's rapid adaptation across a range of climates (Ellstrand & Schierenbeck, 2006; Langevin, Clay, & Grace, 1990). Elucidating the mechanisms by which weedy rice and cultivated rice adapt to low temperatures may provide clues for weedy rice control and the cultivation of high-quality, cold-tolerant rice varieties.

Rice is one of the most globally important staple crops, and numerous studies have been conducted on cold tolerance in this species (Andaya & Mackill, 2003; Cruz Damilach, Kothefederizzi, & Carlos, 2006; Hahn & Walbot, 1989; Ma et al., 2015; Mukhopadhyay, Vij, & Tyagi, 2004; Nakamura et al., 2011; Oh et al., 2004; Saijo, Hata, Kyozuka, Shimamoto, & Izui, 2000; Sakamoto & Murata, 1998; Tao et al., 2011; Wang et al., 2013). Different rice varieties show markedly different levels of cold tolerance, with differential plant survival rates, electrolyte leakage and H_2O_2 contents under low-temperature treatment (Guo Zf, Sy, & Zhong, 2006; Lv et al., 2015; Ma et al., 2015). The cold tolerance of rice populations has been found to be significantly correlated with both latitude and subspecies, with members of the *japonica* subspecies characterized by greater cold tolerance and a wider latitudinal distribution than *indica* varieties (Lv et al., 2015). This *indica–japonica* rice cold tolerance differentiation is also correlated with QTLs and single nucleotide polymorphisms (SNPs) in rice cold-responsive genes. In the abiotic stress response gene *OsMYB2*, two nonsynonymous SNPs in exon 2 (one causing a Cys^{49} to Tyr^{49} change, and the other causing a Trp^{57} to Arg^{57} change) may be involved in *indica–japonica* differences in cold tolerance; *japonica* rice has Cys/Trp, while *indica* accessions have Tyr/Arg at the two sites (Lv et al., 2015). In the fourth exon of *CHILLING TOLERANCE DIVERGENCE* (*COLD1*), a T/C versus A SNP underlies differences of Met^{187}/Thr^{187} in *indica* compared to Lys^{187} in *japonica* varieties; this leads to *japonica* varieties having a greater cold tolerance than *indica* varieties (Ma et al., 2015). More broadly, studies involving quantitative trait locus (QTL) analysis support the idea that cold tolerance is a polygenic trait and that the accumulation of major-effect cold tolerance QTLs can be of considerable value for increasing rice cold tolerance and adaption to high-latitude regions (Andaya & Tai, 2006; Liu et al., 2013; Mao et al., 2015).

Rice responses to low temperatures include cold sensing, transcriptional regulation and post-transcriptional processing. The important roles of calcium, reactive oxygen species (ROS) and abscisic acid (ABA) in cold sensing and signalling have also been revealed. The Ca^{2+}-regulated calcium-regulated protein kinase (CDPK) pathway, ABA-dependent ABRE-binding factor (ABF/AREB) pathway and ROS-dependent mitogen-activated protein kinase (MAPK) pathway all play important roles in signalling transduction in response to low temperatures in rice. Transcriptional regulation includes the dehydration-responsive element-binding proteins-C-repeat/dehydration-responsive elements (*CBF*) pathway and the NAM-ATAF-CUC (OsNAC) transcription factor (Zhang et al., 2013). Post-transcriptional processing mainly involves factors that affect the processing of mRNAs, such as miR-171 and miR-444a (Zhang et al., 2013).

The *CBF* pathway, composed of members of the DREB (dehydration-responsive element-binding) gene family and their regulators, is the best documented and most extensively characterized cold response pathway to date (Agarwal, Agarwal, Reddy, & Sopory, 2006; Dubouzet et al., 2003; Ito et al., 2006; Ma et al., 2009; Qin et al., 2007; Su et al., 2010; Thomashow, 1999, 2010; Yang, Dai, & Zhang, 2012; Zhang, Chen, Wang, Hong, & Wang, 2014). These TFs can bind to GCC-box and C-repeat/ dehydration-responsive elements (CRT/DRE) to promote the expression of cold-responsive genes (Zhang et al., 2013). *CBF1* and *CBF3* play important roles in increasing plants' cold tolerance. *CBF2* functions in a complementary role as a negative regulator of *CBF1* and *CBF3*, ensuring that their expression is transient and tightly controlled, which in turn guarantees the proper induction of downstream genes (Novillo, Alonso, Ecker, & Salinas, 2004). *OsMYB2* encodes a stress-responsive *MYB* transcription factor that promotes the expression of *CBFs* (Zhang et al., 2014). Inducer of *CBF* expression 1 (*ICE1*) is another important regulator of *CBF* pathway genes that promotes their expression by binding to MYC recognition sites in the *CBF* promoter regions. Levels of expression of *CBF* pathway genes are known to be correlated with cold tolerance, with significantly higher relative expression found in cold-tolerant cultivated rice and weedy rice than in cold-sensitive populations (Bevilacqua et al., 2015). However, the mechanisms underlying the differential expression of *CBF* pathway genes have not yet been fully elucidated. A recent study has suggested that cold tolerance variation in rice may be related to methylation of the *CBF* regulator *OsICE1* (Han, Li, Xie, Dai, & Qiang, 2017). Thus, methylation-mediated epigenetic control of gene expression could be an important mechanism underlying cold response variation in cultivated rice and co-occurring weedy rice.

DNA methylation, an important form of epigenetic regulation, is broadly involved in gene expression regulation and stress response during growth and development (Chinnusamy, 2009; Kawakatsu et al., 2016; Sanchez & Paszkowski, 2014; Urano, Kurihara, Seki, & Shinozaki, 2010; Zilberman, Gehring, Tran, Ballinger, & Henikoff, 2007). In plants, DNA methylation occurs

at three distinct site classes: CG, CHG and CHH (where H is A, C or T). Cytosine methylation in the promoter region tends to negatively regulate gene expression (Smith & Meissner, 2013; Zhang et al., 2006). Methylomic studies have shown that the methylation level of CG sites is positively correlated with gene expression levels (Wang et al., 2015); however, other studies have not observed a correlation with gene expression (Jones, 2012). Epigenomic studies in *A. thaliana* have suggested that CHH methylation increases with growth temperatures while CG methylation is instead correlated with the climate of origin (Dubin et al., 2015). However, most current studies characterizing DNA methylation are based on DNA methylomic analyses (Kawakatsu et al., 2016; Zilberman et al., 2007), and few studies have directly examined the methylation of individual genes.

In a previous study, we found that methylation of the *ICE1* coding region, including CG, CHG and CHH sites, is correlated with cold tolerance in crofton weed (*Ageratina adenophora*), which could potentially contribute to its rapid invasion northward in China (Xie et al., 2015). Few studies have been conducted on the role of DNA methylation in rice cold tolerance (Dai et al., 2015; Pan et al., 2011). Moreover, the DNA methylation of the *CBF* pathway genes has not been examined in relation to the cold tolerance of weedy rice. Since weedy rice is physiologically and morphologically similar to rice, assessing CBF gene methylation in relation to cold tolerance variation of different geographical populations of weedy rice and rice may help to reveal the mechanisms underlying their parallel adaptation to local climates. Studying the expression of *CBF* pathway genes of different cold-tolerant weedy rice and rice populations can further elucidate the molecular mechanism underlying cold tolerance codifferentiation. To address these topics, we assessed the cold tolerance of 100 weedy rice populations and co-occurring rice varieties across China and investigated whether DNA methylation of the *OsICE1* gene is involved in cold tolerance regulation.

2 | MATERIALS AND METHODS

2.1 | Sampling and geographical information collection

A total of 100 weedy rice and 100 associated rice populations were collected in 100 sites across 23 provinces in China, including Heilongjiang, Ningxia, Jiangsu, Guangdong and Hainan provinces, between 2006 and 2016 (Table S1). Seeds of more than 20 weedy rice individuals were collected at each sampling site, with sampling spaced at 20-m intervals. Since cultivated rice plants were typically a single variety within each sampling site, seeds of only one rice individual were collected per site. Seeds of each sampled individual were stored in paper bags, air-dried at ambient temperature and then stored at −20°C. After the weedy rice and rice seeds were collected from the field, the seeds were grown in a common garden environment in Nanjing, China (118°37′E, 32°02′N). The seeds produced in the common environment were used as the materials for the experiment. The latitude, longitude and altitude of each sampling location were recorded using the GPS recorder eTrex (Garmin International 1200 E. 151 St. Dock Door #24. Olathe, KS 66062). Climatic information for each sampling site was calculated using data between 2006 and 2016 from the China Meteorological Network (http://data.cma.cn/site/index.html).

2.2 | Cold tolerance determination of weedy rice and rice populations

Seeds of weedy rice and rice were heat-treated at 50°C for 48 hr to eliminate seed dormancy. Then, the seed surfaces were sterilized with 70% ethanol for 30 s and washed with sterile water five times. The seeds were soaked in water overnight and germinated in the dark for 4 days before being transplanted to 10-cm-diameter pots. Pots were maintained at 28°C in a growth chamber with a 12/12-hr photoperiod, 300 μmol m^{-2} s^{-1} fluorescent lighting and 70%–80% relative humidity. For each sampling site, seeds from five weedy rice individuals and the cultivated rice individual were planted; four pots with three seeds per pot were planted for each individual. At the four-leaf stage, three of the pots of seedlings of each accession were placed at 5°C for 3 days and then returned to a 28°C growth chamber for another 3 days (300 μmol m^{-2} s^{-1}, 12/12-hr photoperiod, 70%–80% relative humidity). The fourth pot of seedlings was placed at 28°C for 6 days as a control. The cold injury indices of the seedlings were determined as previously described using the 1-cold injury index (Li, Qiang, & Qian, 2008). After seedlings were treated at 5°C for 3 days, photosynthetic parameters of the weedy rice and rice populations were determined using an Imaging-PAM fluorometer (Heinz Walz GmbH; Ehlert & Hincha, 2008). Seeds of five weedy rice individuals and one cultivated rice individual were also used for photosynthetic parameter determination. Each individual, represented by three leaves from three different seedlings, was assessed for photosynthetic parameters including the effective quantum yield of photosystem II (Y(II)), electron transport rate (ETR1) and photochemical quenching (qP); the experiment was conducted in triplicate for each individual. The plants were dark-adjusted for 30 min before measurement, and the distance between the leaf and charge-coupled device (CCD) camera was set to 18 cm. The instrument settings and parameters were set as described previously (Schreiber, Walz, & Kolbowski, 2003), and the saturation pulse intensity was set to 13.

2.3 | Quantitative real-time PCR (qPCR) analysis of CBF pathway genes

The expression levels of five *CBF* pathway genes were determined by qPCR analysis of three weedy rice populations (WRLN004, WRJS023 and WRGD008) and their associated rice populations

(WRLN004R, WRJS023R and WRGD008R, respectively) that together represent the phenotypic range of cold tolerance observed in the study. The five targeted genes were *OsICE1*, *OsCBF1*, *OsCBF2*, *OsCBF3* and *OsMYB2*. As described above, five weedy rice individuals and one cultivated rice individual were used for qPCR analysis. Eight seedlings of each individual were treated at 5°C for 0, 0.5, 1, 2, 4, 8, 12 and 24 hr, and 0.1 g of leaves from each treatment was used for RNA isolation using RNAiso for Polysaccharide-rich Plant Tissue (TaKaRa Bio Inc. Setu 3-4-1, Otsu, Shiga 520-2193, Japan) according to the manufacturer's protocol. The cDNA was prepared using PrimeScript™ RT Reagent Kit with gDNA Erase (TaKaRa). The reaction mixture (20 μl) contained 10 μl of SYBR® *Premix* Ex Taq™ (2×; TaKaRa), 2 μl of primers (Table S2), 1 μl of cDNA and 7 μl of ddH_2O. The reaction conditions for qPCR were as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. The fold changes in the *CBF* pathway genes, which were normalized to beta-actin, were calculated for each population using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). Three biological replicates were conducted for each gene, and three different seedlings were used for qPCR analysis in each treatment.

2.4 | Methylation determination

After seedlings of the five weedy rice individuals and one rice individual were treated at 5°C for 3 days and cultured at 28°C for another 3 days, 0.1 g of leaves from each individual was used for DNA isolation using a Plant Genomic DNA Kit (TIANGEN Biotech (BEIJING) CO, LTD. Building 1-604, Hangxing, No. 27 Long 258 Caoxi Road Xuhui District, Shanghai, China). The methylation sites in the *OsICE1* gene of three weedy rice populations (WRLN004, WRJS023 and WRGD008) and their associated rice populations (WRLN004R, WRJS023R and WRGD008R) were determined using bisulphite modification. The *OsICE1* promoter (1,000 bp upstream of ATG) of 100 weedy rice populations and their associated rice populations was also determined. Up to 450 ng of genomic DNA was treated using an EZ DNA Methylation-Gold™ Kit (ZYMO Research) following the manufacturer's guidelines. Then, PCR amplification was performed using EpiTaq™ HS (for bisulphite-treated DNA; TaKaRa) under the following conditions: 10 s at 98°C; 35 cycles of 10 s at 98°C, 1 min at 52°C and 1 min at 72°C; and a final extension for 10 min at 72°C. Each reaction mixture contained 5 μl of 10× EpiTaq PCR Buffer (Mg^{2+} free), 6 μl of a dNTP mixture, 5 μl of $MgCl_2$ (25 mM), 2.0 μl of primers (10 μM; Table S3), 1.25 U of EpiTaq HS, 100 ng of DNA and sterilized distilled water (to a final volume of 50 μl). The PCR product was ligated to the pMD™19-T vector (pMD™19-T Vector Cloning Kit, TaKaRa), and 10 clones were sequenced for each sample. The obtained sequences (GenScript USA Inc.) of each sample were compared with the original *OsICE1* sequence, and a converted cytosine indicated an unmethylated site. The thresholds for labelling a cytosine as methylated in the CG, CHG or CHH contexts (where H is A, C or T) were set to 80%, 50% and 50%, respectively (Cokus et al., 2008). *OsICE1* methylation determination was conducted three times for each individual.

2.5 | Data analysis

Correlation analysis was performed to test for associations between the geographical and climate information (latitude, altitude, annual average temperature, maximum temperature, minimum temperature, June average temperature), cold tolerance data, single nucleotide polymorphisms (SNPs) of the *OsICE1* promoter and *OsICE1* methylation patterns of the different weedy rice and rice populations; analyses were conducted using SPSS 20 software (IBM SPSS Statistics 20), with the Pearson index as the correlation coefficient. Differences in the relative expression levels of the five *CBF* pathway genes for weedy and cultivated rice populations were analysed by one-way analysis of variance (ANOVA; IBM SPSS Statistics 20) with the least significant difference (LSD) multiple comparison method. These data were plotted by Origin 9 (OriginLab Corporation). The geographical distributions of the cold tolerances and *OsICE1* methylation states of the different weedy rice and rice populations were plotted by ArcMap 10 (Environment Systems Research Institute). Differences in *OsICE1* methylation levels of CHG and CHH at different latitudes were compared by independent sample *t* tests (SPSS 20). The boxplots were plotted by Origin 9.

3 | RESULTS

3.1 | Cold tolerance variation in weedy and cultivated rice populations

Sampled weedy rice and cultivated rice accessions varied widely in their response to the cold treatment (5°C for 3 days; see Section 2), and accessions were classified into three categories according to their cold tolerance abilities: cold-tolerant populations (cold tolerance ≥ 0.58), intermediately cold-tolerant populations (0.20 ≤ cold tolerance < 0.58) and cold-sensitive populations (cold tolerance < 0.20). Representative weedy rice and cultivated rice phenotypes for the three cold tolerance categories are shown in Figure 1. Cold-sensitive plants were nearly dead after cold treatment (Figure 1a,b). Intermediately cold-tolerant plants lost greenness in their leaves, and their growth was significantly inhibited (Figure 1c,d). Cold-tolerant plants showed minimal visible signs of damage, with only a small portion of the leaves curled after cold treatment and no significant differences evident from the untreated control seedlings (Figure 1e,f).

For all plants tested, the cold treatment significantly decreased photosynthetic parameters, including the effective quantum yield of photosystem II (Y(II)), electron transport rate (ETR1) and photochemical quenching (qP). However, as with the cold injury index,

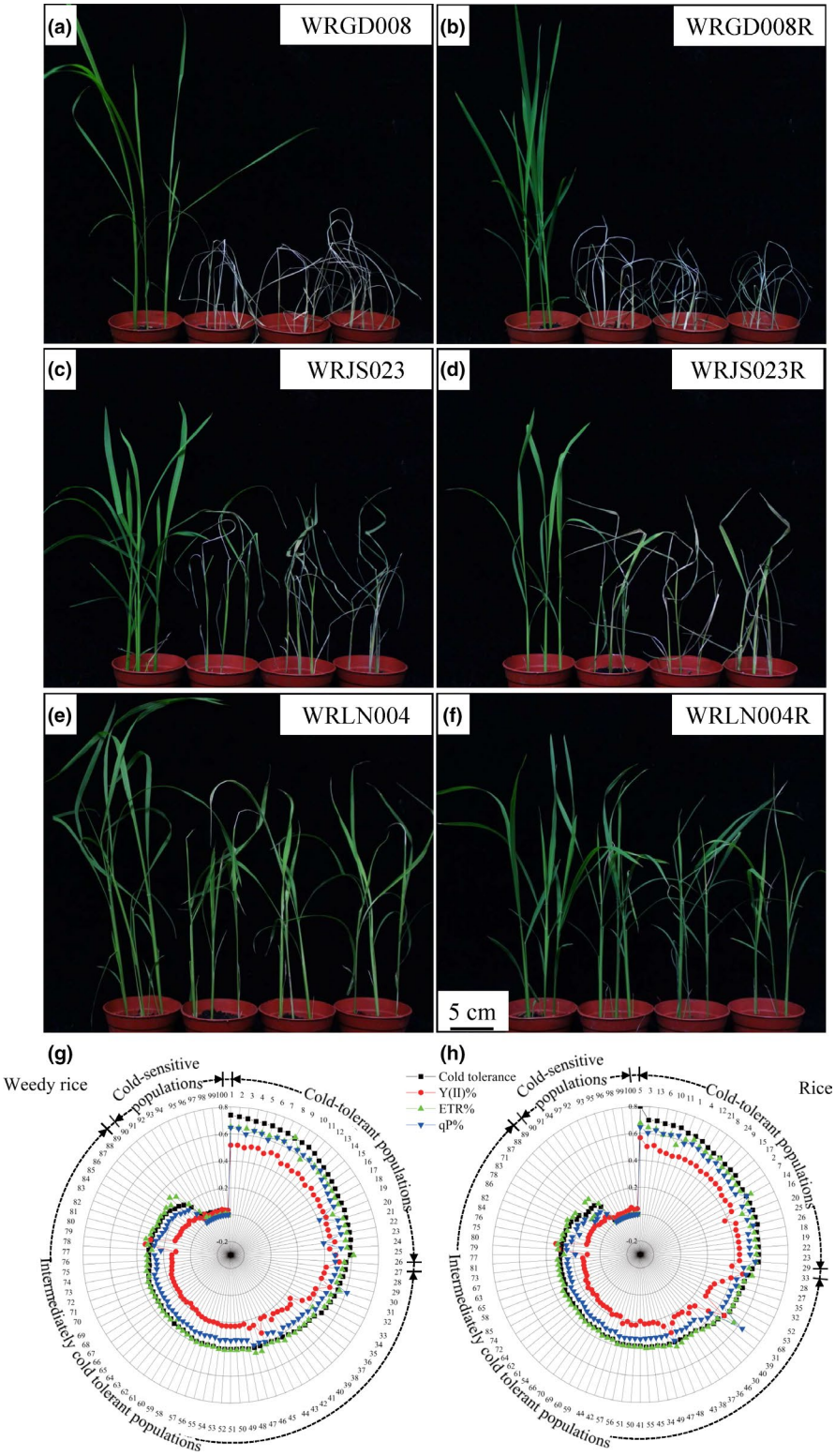

FIGURE 1 Phenotypic changes, cold tolerance and photosynthetic parameters of weedy rice and rice after cold treatment. The population numbers are listed in Table S1

the level of decrease varied widely among samples from different climates (Figure 1g,h). The Y(II), ETR1 and qP values of the cold-tolerant weedy rice populations decreased by 48%–56%, 30%–48% and 35%–60%, respectively, after cold treatment for 3 days. For the intermediate cold-tolerant weedy rice populations, these photosynthetic parameters were decreased by 49%–92%, 45%–72% and 39%–84%, respectively. For the cold-sensitive weedy rice populations, the photosynthetic parameters were decreased by 94%–97%, 81%–100% and 90%–100%, respectively. The cold-sensitive populations showed the most significant decrease in fluorescence parameters compared with those of the intermediately cold-tolerant and cold-tolerant populations, indicating that cold temperatures caused more serious damage to the photosynthetic reaction centre than that exhibited by the other populations.

3.2 | Cold tolerance is correlated with sampling site locations

For both weedy rice and co-occurring cultivated rice varieties, cold tolerance levels were strongly correlated with geographical and climatic data for the sampling sites. For both groups, there were significant positive correlations between cold tolerance and latitude (Figure 2a) and significantly negative correlations with average annual minimum temperature (Figure 2b), average annual temperature (Figure 2c) and June average temperature (Figure 2d). Interestingly, while the weedy rice and cultivated rice populations from different collection sites showed significant cold tolerance variation, weedy and cultivated individuals from a given sampling site showed no significant differences from each other ($p < .05$; Figure 1g,h). Thus, the weedy rice and associated cultivated rice showed parallel patterns of cold tolerance across the different sampled populations. For both weedy rice and cultivated rice, cold tolerance was not correlated with altitude (Figure 2e) or annual maximum temperatures (Figure 2f) of the sampling sites.

Cold tolerance variation was broadly correlated with geographical regions of rice cultivation across China. The high-latitude populations, represented by 26 weedy rice populations and 27 cultivated rice varieties, were all in the cold-tolerant category. These populations were located in Heilongjiang, Liaoning, Xinjiang, Ningxia, Gansu and Shanxi provinces at latitudes ranging from 37° to 48°. These northern, cold-tolerant populations occur in regions with low annual average temperatures, ranging from 1 to 12°C. The high-latitude locales are also characterized by low annual minimum temperatures (−37 ~ −23°C) and cool June average temperatures (16 ~ 19°C). The intermediately cold-tolerant populations, comprising 63 weedy rice populations and 62 rice populations, were located in central and central-southwest China. These mid-latitude regions (latitudes ranging from 21° to 39°) serve as the main area for rice cultivation in China and include the Hebei, Shandong, Jiangsu, Shanghai, Anhui, Henan, Sichuan, Guizhou, Yunnan and Guangxi provinces. These areas have subtropical or warm temperate climates and relatively higher annual average temperatures, ranging from 11 to 23°C, with minimum temperatures in the range of −20 to 1°C and June average temperatures ranging from 19 to 25°C. The low-latitude populations (latitudes ranging from 18° to 21°), which were represented by 11 weedy rice and co-occurring cultivated rice populations, were all cold-sensitive. These regions have tropical or southern subtropical monsoon climates and high annual average temperatures ranging from 20 to 27°C, the warmest annual minimum temperatures (−6 ~ 9°C) and the warmest June average temperatures (25 ~ 28°C).

3.3 | The CBF pathway plays a role in the cold tolerance of weedy rice and rice populations

The qPCR analysis showed that the expression levels of *OsICE1*, *OsCBF1*, *OsCBF2*, *OsCBF3* and *OsMYB2* were correlated with the cold tolerances of the tested weedy rice and associated cultivated rice plants (Figure 3). For all five genes, expression was low for all plants in the control treatment (0 hr), with no significant differences among accessions. However, expression increased over successive time points of the cold treatment, with significant differences emerging between the plants with different levels of cold tolerance. For the *CBF* regulator *OsICE1*, no differences in the expression levels were observed among the different populations before 2 hr of cold treatment (Figure 3a). After 4–8 hr of cold treatment, the expression levels in the cold-tolerant WRLN004 and WRLN004R populations were significantly higher than those in the intermediately cold-tolerant and the cold-sensitive populations. After 12–24 hr of cold treatment, WRLN004 and WRLN004R had the highest *OsICE1* expression levels, while the cold-sensitive WRGD008 and WRGD008R populations had the lowest *OsICE1* expression levels. Throughout the entire cold treatment process, the *OsICE1* expression level showed no significant difference between weedy rice and the co-occurring cultivated rice, consistent with their shared levels of cold tolerance.

For *OsCBF1*, the different cold-tolerant weedy rice and rice populations showed no significant difference in expression levels before 1 hr of cold treatment (Figure 3b). After 1 hr of cold treatment, the different cold-tolerant weedy rice populations varied significantly in *OsCBF1* expression levels, while the cultivated rice populations showed no difference. After 4–24 hr of cold treatment, the cold-tolerant WRLN004 and WRLN004R populations exhibited the highest *OsCBF1* gene expression levels, while the cold-sensitive WRGD008 and WRDD008R populations exhibited the lowest expression levels. As with *OsICE1*, the difference among the different cold-tolerant populations increased as the cold treatment process continued.

Expression patterns for *OsCBF3* and *OsMYB2* were broadly similar to *OsICE1* and *OsCBF1* over the cold treatment time-course. *OsCBF3* expression in the different cold-tolerant populations gradually increased under cold treatment and peaked at 12 hr of cold treatment (Figure 3d). The weedy rice and rice populations continued to exhibit high *OsCBF3* gene expression levels after 4 hr of cold treatment, and the cold-tolerant WRLN004 and WRLN004R plants exhibited higher expression levels than the others. For *OsMYB2*, there was no significant difference or only a small difference in the *OsMYB2* gene expression levels among the different weedy rice and rice populations before 4 hr of cold treatment (Figure 3e). After 8 hr of cold treatment, the cold-tolerant WRLN004 and WRLN004R populations showed significantly higher *OsMYB2* expression levels than the others. Throughout the entire cold treatment process, weedy rice generally had the same *OsMYB2* gene expression level as its associated cultivated rice population.

OsCBF2 encodes a negative regulator of the *CBF* pathway, and its expression showed a decline from 2–24 hr that was coincident with the increasing expression of the other genes (Figure 3c). At 1–2 hr of cold treatment, the weedy rice and rice populations all had relatively high *OsCBF2* gene expression levels. Weedy rice generally had higher expression levels than its associated rice populations. However, there were no significant differences among the different cold-tolerant weedy rice and rice populations. After 4–12 hr of cold treatment, the *OsCBF2* gene expression levels decreased gradually, and the

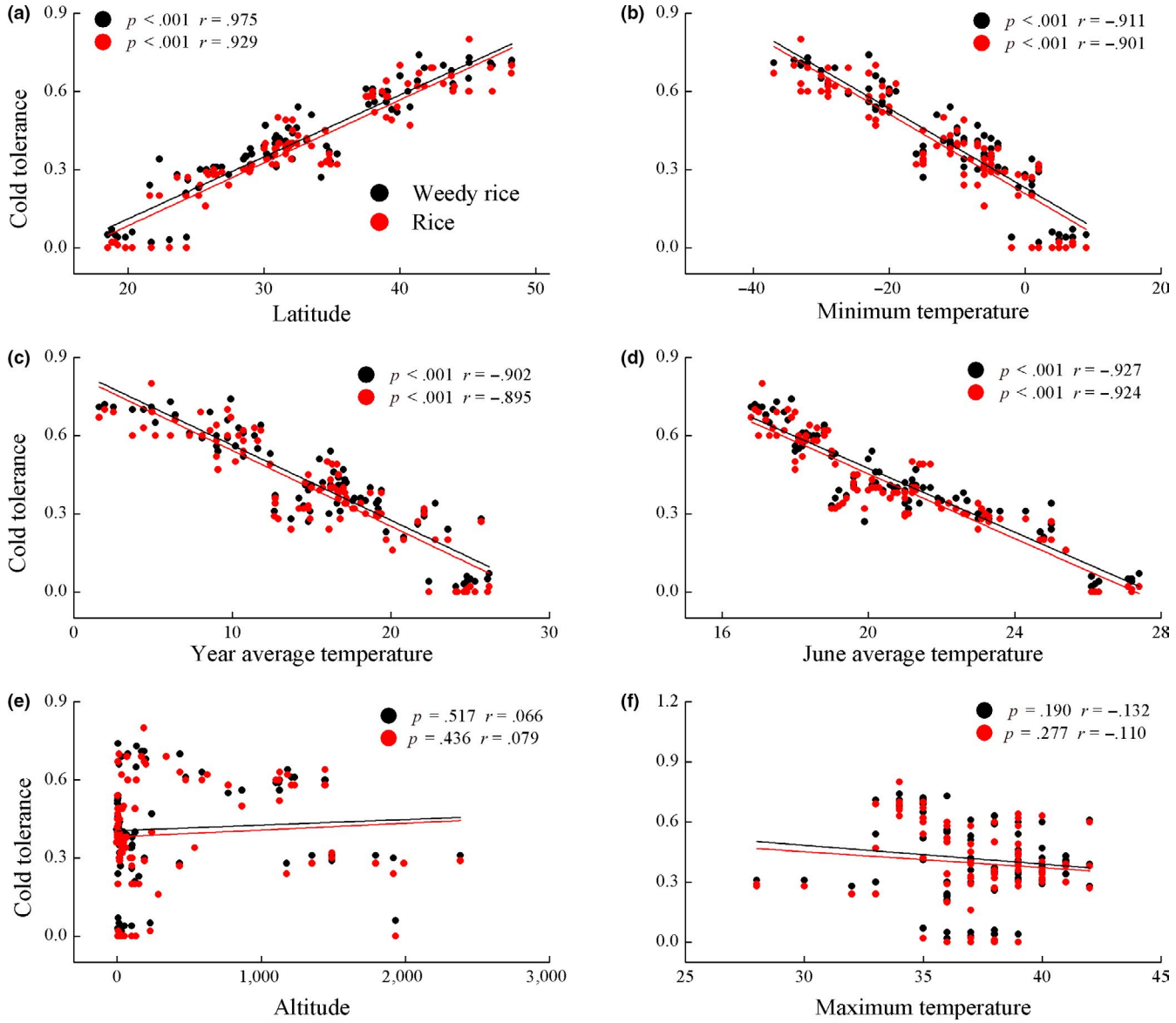

FIGURE 2 Correlation analysis of cold tolerance and geographical information [Colour figure can be viewed at wileyonlinelibrary.com]

cold-tolerant WRLN004 and WRLN004R populations decreased more rapidly than the others. This led to higher expression levels of this *CBF* negative regulator in the cold-sensitive WRGD008 and WRGD008R accession than in the cold-tolerant and intermediate cold-tolerant populations. The weedy rice and rice populations all continued to exhibit low *OsCBF2* gene expression levels after 24 hr of cold treatment.

In summary, the *CBF* pathway genes of different cold-tolerant weedy rice and rice populations showed no significant differences at the initial stage of cold treatment, but the difference gradually increased as the cold treatment progressed. Except for the negative regulator, *OsCBF2*, the expression levels of *CBF* pathway genes were positively correlated with cold tolerance. In accordance with the similar cold tolerances of weedy rice and its associated rice population, there was no significant difference or only a slight difference in the *CBF* pathway gene expression levels between the weedy rice samples and associated cultivated rice varieties. These patterns indicate that *CBF* pathway genes play a role in the shared patterns of cold tolerance in weedy rice and cultivated rice populations.

3.4 | OsICE1 promoter methylation is correlated with cold tolerance in weedy rice and cultivated rice

Whereas the coding regions of *OsICE1* were not methylated in three sampled weedy rice populations and their associated rice populations (see data in Dryad archive), methylation was present in the promoter region, and weedy rice shared the same DNA sequence in this promoter region with its associated cultivated rice. There were six SNPs in *OsICE1* promoter region (-670C/deletion, -546 C/deletion, -463 deletion/C, -420 deletion/C, -404C/A and -392C/G). However, these SNPs were not correlated with cold tolerance (Table S4). Overall, the total numbers of methylated sites in the *OsICE1* promoter were 22–33

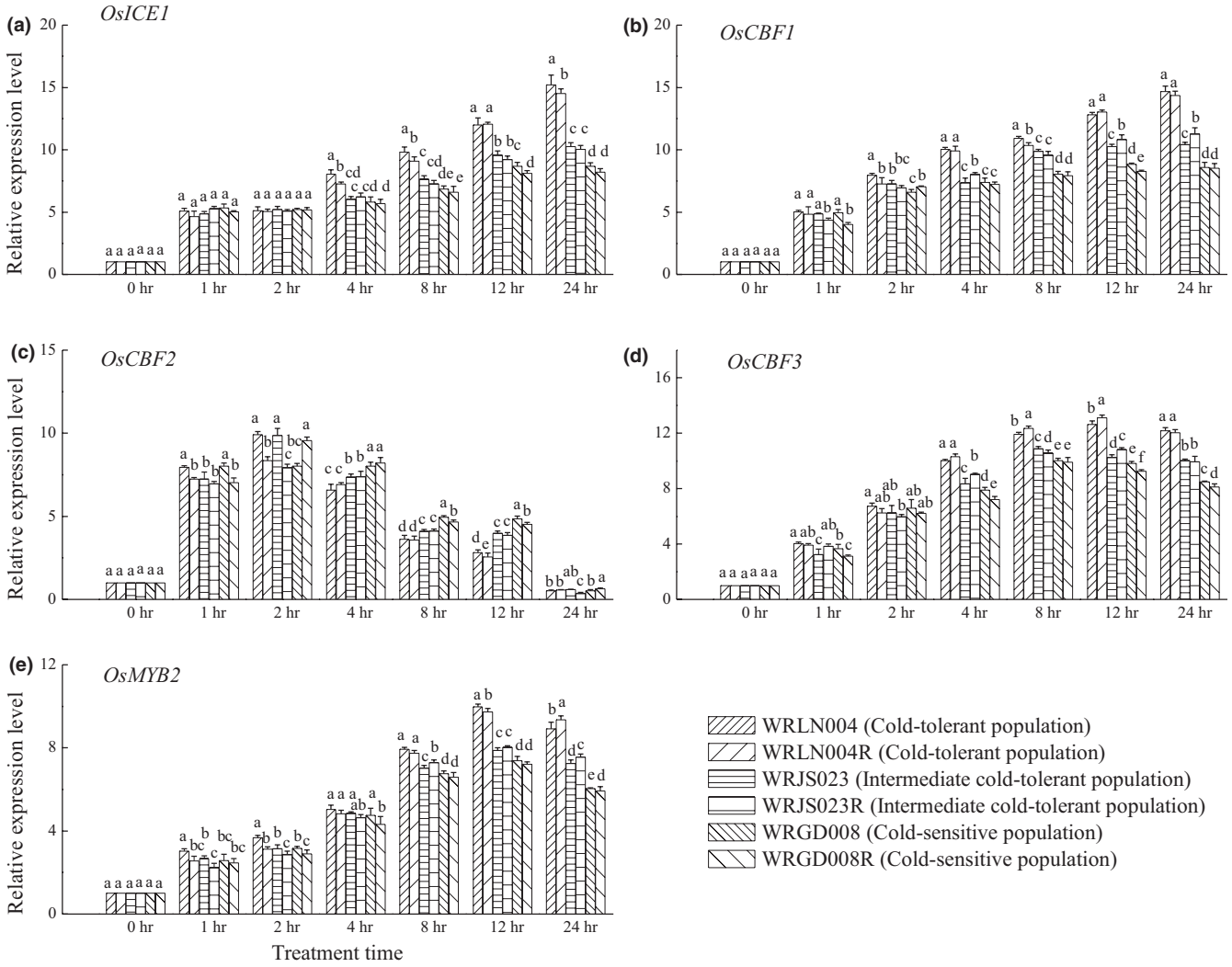

FIGURE 3 *CBF* pathway gene expression levels in different cold-tolerant weedy rice populations and their associated rice populations. The letters indicate significant differences among the different populations, $p < .05$

and 21–35 for weedy rice and cultivated rice, respectively. Weedy rice had 19–23 CG methylation sites, 1–7 CHG methylation sites and 0–5 CHH methylation sites, while cultivated rice had 15–24 CG methylation sites, 1–7 CHG methylation sites and 0–7 CHH methylation sites (Table S1). Among the 100 geographical locations sampled, 68 were characterized by having the same methylation levels in weedy rice samples as in the co-occurring cultivated rice. For the remaining locales, methylation levels in the weedy rice populations were only slightly different from those in their associated rice populations (Figures 4, S1 and S2). Thus, throughout the sampled geographical range, weedy and cultivated rice from a given locale shared identical or similar *OsICE1* promoter methylation patterns.

The *OsICE1* promoter methylation levels in the weedy rice and cultivated rice populations were significantly negatively correlated with cold tolerance and the climates of the sampling sites, with plants from colder climates showing low *OsICE1* promoter methylation levels and those from warmer climates showing high methylation levels. Consistent with this pattern, the *OsICE1* promoter methylation levels in the representative cold-tolerant (WRLN004 and WRLN004R), intermediate cold-tolerant (WRJS023 and WRJS023R) and cold-sensitive (WRGD008 and WRGD008R) weedy rice and rice populations were significantly negatively correlated with *OsICE1* gene expression level (Figure 5). Similarly, the promoter methylation levels were also significantly negatively correlated with expression levels of other *CBF* pathway genes (Figure S3). These patterns held true when considering all *OsICE1* promoter methylation sites (Figure 5a) and for CHG and CHH methylation sites specifically (Figure 5b), although not for CG methylation level as the three typical cold-tolerant weedy rice and rice cultivars all had 20 CG methylation sites in the *OsICE1* promoter (Table S1). For both weedy rice and cultivated rice, the *OsICE1* promoter methylation level was positively correlated with the minimum temperature, annual average temperature and June average temperature of collection locations (Figure 6). These same correlations were also apparent for CHG and CHH methylation levels individually (Figure 7), but not for CG methylation levels (Figure S4).

The CG methylation sites on the *OsICE1* promoters of weedy rice and rice populations were highly conserved, as the methylation sites were essentially the same between weedy rice populations and co-occurring rice varieties. The CHG methylation sites were also

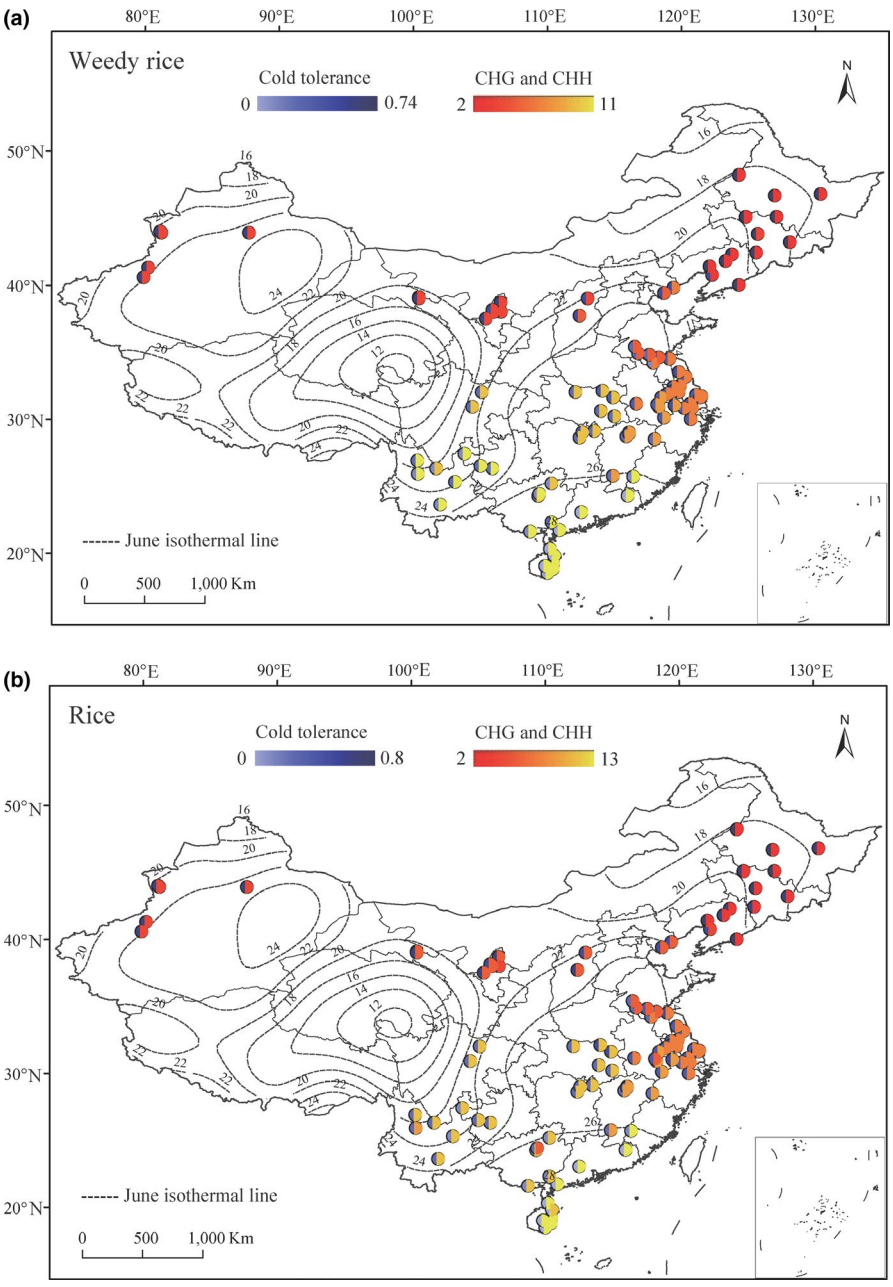

FIGURE 4 Distribution, cold tolerance and CHG and CHH methylation levels of the *OsICE1* promoter in China

relatively conserved. Most of the CHG methylation sites between the weedy rice and rice populations were the same and occurred mostly between −385 and −574 bp. Compared with the CG and CHG methylation sites, the diversity of the CHH methylation sites was significantly increased. Only some of the methylation sites were conserved between the weedy rice and rice populations (Figure 8).

3.5 | CHG and CHH methylation play different roles in cold tolerance differentiation at different latitudes

At similar latitudes, there were also regional differences in levels of cold tolerance that were correlated with methylation patterns. In the high-latitude regions, where the annual average temperature was approximately 20°C, the weedy rice and cultivated rice populations of the Northeast (Heilongjiang, Jilin and Liaoning) had higher cold tolerance than those of the northwest (Xinjiang, Ningxia, Shanxi and Gansu). These regional differences in cold tolerance were correlated with the CHG methylation levels of the *OsICE1* promoter (Figures 9a and S5a), but not with the CHH methylation levels (Figure S5b). Both weedy and cultivated rice populations in Northeast China had only one CHG methylation site on the *OsICE1* promoter with few exceptions. The northwest weedy rice and cultivated rice populations had 4–5 and 3–5 CHG methylation sites, respectively.

The mid-latitude regions (including Hebei, Shandong, Henan, Jiangsu, Anhui, Shanghai, Hubei, Jiangxi, Hunan, Zhejiang, Sichuan, Guizhou, Yunnan and Guangxi) serve as the main area for rice cultivation and have an annual average temperature of 24°C. However,

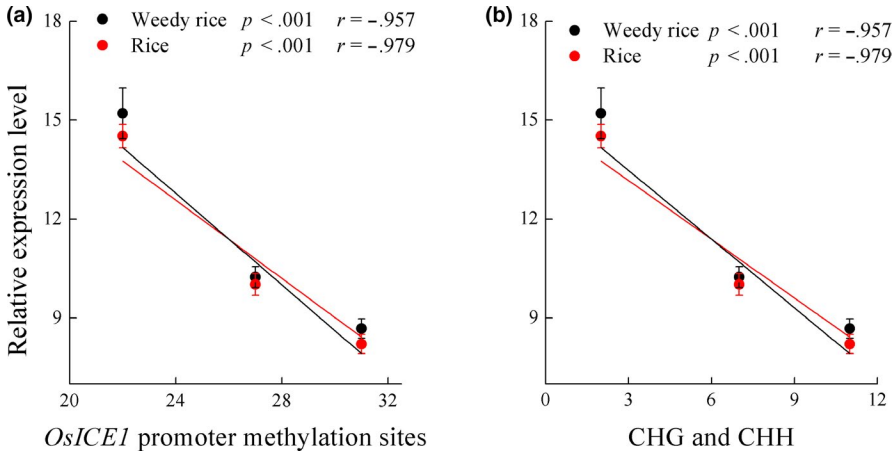

FIGURE 5 Correlation analysis of the *OsICE1* promoter and gene expression levels among the representative cold-tolerant weedy rice and rice populations [Colour figure can be viewed at wileyonlinelibrary.com]

the cold tolerances of weedy rice and rice in these areas also varied, decreasing significantly from the central northeast to the central northwest (Figure S5c,d). Interestingly, in contrast to high-latitude regions, differences in both CHG and CHH methylation levels at the *OsICE1* promoter contributed to the cold tolerance differentiation between these mid-latitude regions (Figure 9c,d). The central northwest populations had higher CHG and CHH methylation levels than the central northeast populations. The weedy rice and rice populations in the central northeast, including the Hebei, Shandong, Jiangsu, Anhui, Shanghai and Zhejiang populations, had 4–7 CHG methylation sites and 1–3 and 0–3 CHH methylation sites, respectively. The central northwest weedy rice and rice populations in Henan, Hubei, Jiangxi, Hunan, Sichuan, Guizhou, Yunnan and Guangxi had 4–6 and 3–5 CHG and CHH methylation sites, respectively (Figure S5c,d).

The temperatures in low-latitude regions, including Fujian, Guangdong and Hainan, are relatively high year around with an annual average temperature of 28°C. The weedy rice and rice populations in these regions were universally cold-sensitive, and little difference in cold tolerance was observed among these populations (Figure S5e,f). Consistent with this observation, the weedy rice and cultivated rice populations all had similar CHG and CHH methylation levels, with 6–7 CHG methylation sites and 3–5 and 3–7 CHH methylation sites, respectively (Figure 9e,f). Compared with the high-latitude and mid-latitude populations, the relatively high CHG and CHH methylation levels in the low-latitude populations is consistent with the low cold tolerance observed in this subtropical to tropical region.

4 | DISCUSSION

The cold tolerances of different cultivated rice varieties and their associated weedy rice populations differed substantially across China, and cold tolerance was closely correlated with the local climate (Figure 2). Cultivated rice is the product of artificial selection, and only the rice strains that perform well at local temperatures would be expected to be incorporated into the local crop germplasm. Rice frequently encounters low-temperature stress during the sowing period, especially in temperate regions, and the cold tolerance of rice is essential for local adaptation. Increased cold tolerance in rice appears to come at an energetic cost, however, as it reduces rice yield in tropical and southern subtropical regions where low-temperature stress rarely occurs (Mitra & Bhatia, 2008; Morin et al., 2007; Stoks & De Block, 2011). Therefore, cold-tolerant rice strains are unlikely to be selected in these warmer areas.

A notable finding of this study is the very close similarity in levels of cold tolerance between local cultivated rice varieties and their co-occurring weedy rice populations (Figure 2). Unlike cultivated rice, weedy rice has invaded rice production areas despite attempts to keep it out, and it has adapted to local climates without the benefits of deliberate artificial selection. While some weedy rice strains are direct descendants of the modern cultivars whose fields they infest (e.g., some Malaysian weedy rice strains; Song et al., 2014), the majority are not, and recent whole-genome resequencing studies suggest that some contemporary weed strains originated early in the history of rice cultivation (Li et al., 2017). The ability of weedy rice to adapt across such a wide range of climates as were sampled in this study explains why weedy rice can occur in a broad range of environments naturally. This adaptability has undoubtedly played a role in the widespread occurrence of weedy rice in most of the world's rice production areas, including Asia, Europe, Africa, North America and South America (Wedger & Olsen, 2018).

The cold tolerances of the weedy rice and rice populations were correlated with local temperatures, with the more northeastern populations (corresponding to those at higher latitudes) having higher cold tolerances than the southwestern populations. This pattern corresponds to the lower minimum temperatures in the northeast regions (Table S1) and accounts for the significant correlations between cold tolerance and latitude (Figure 6). This regional variation also corresponds to variation in the predominant rice subspecies that is cultivated locally. In China, *japonica* rice tends to be grown at higher latitudes while *indica* rice is grown in warmer, lower-latitude regions. Similarly, *japonica*-like weedy rice occurs more in cooler, northern regions, and *indica*-like weeds predominate at lower latitudes. In general, *japonica* populations have a higher cold tolerance than *indica* populations (Lv et al., 2015). This provides a basis for the selection of rice varieties in China, and *japonica* varieties are preferred in high-latitude, low-temperature areas.

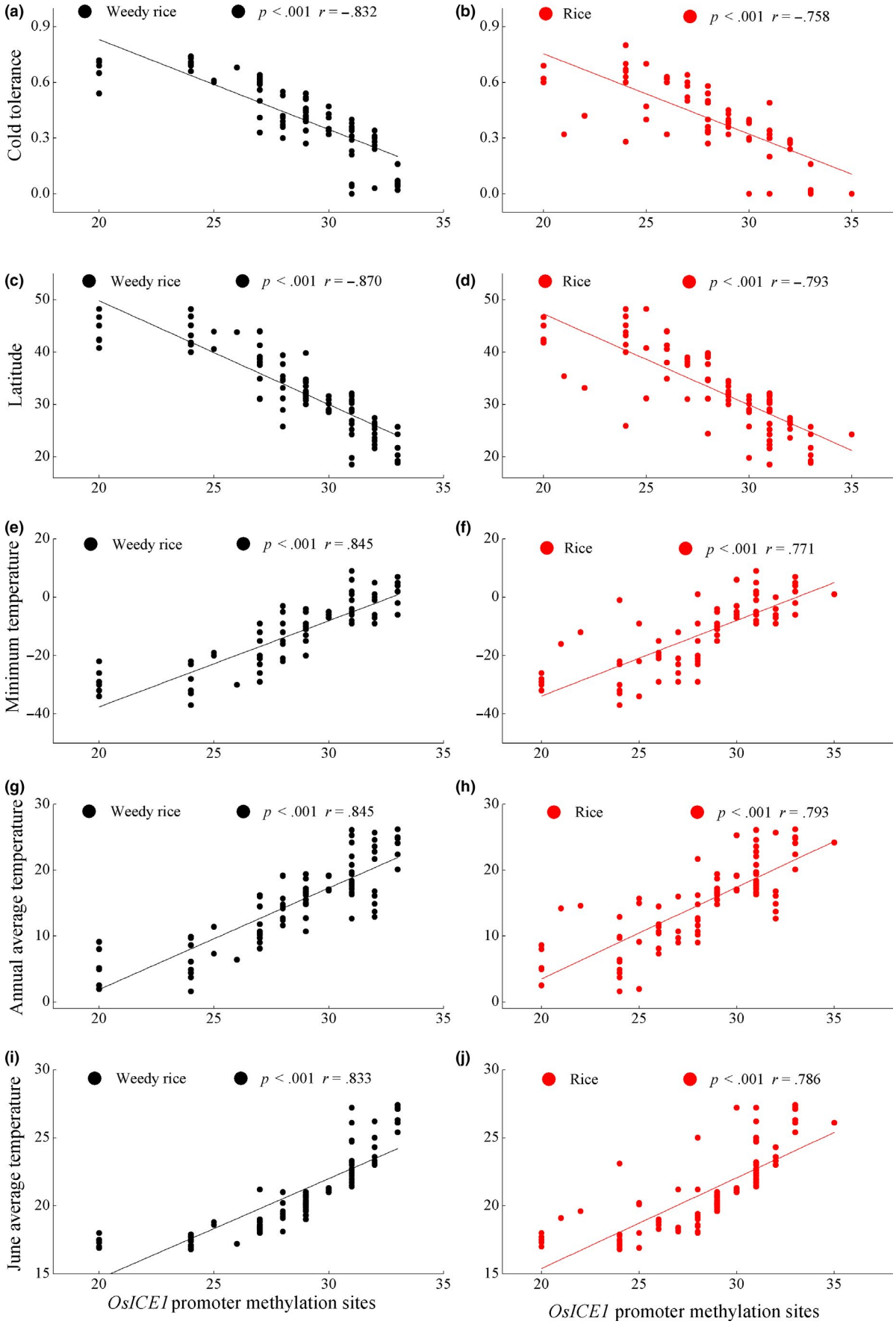

FIGURE 6 Correlation analysis of the *OsICE1* promoter methylation level and the cold tolerance and geographical information [Colour figure can be viewed at wileyonlinelibrary.com]

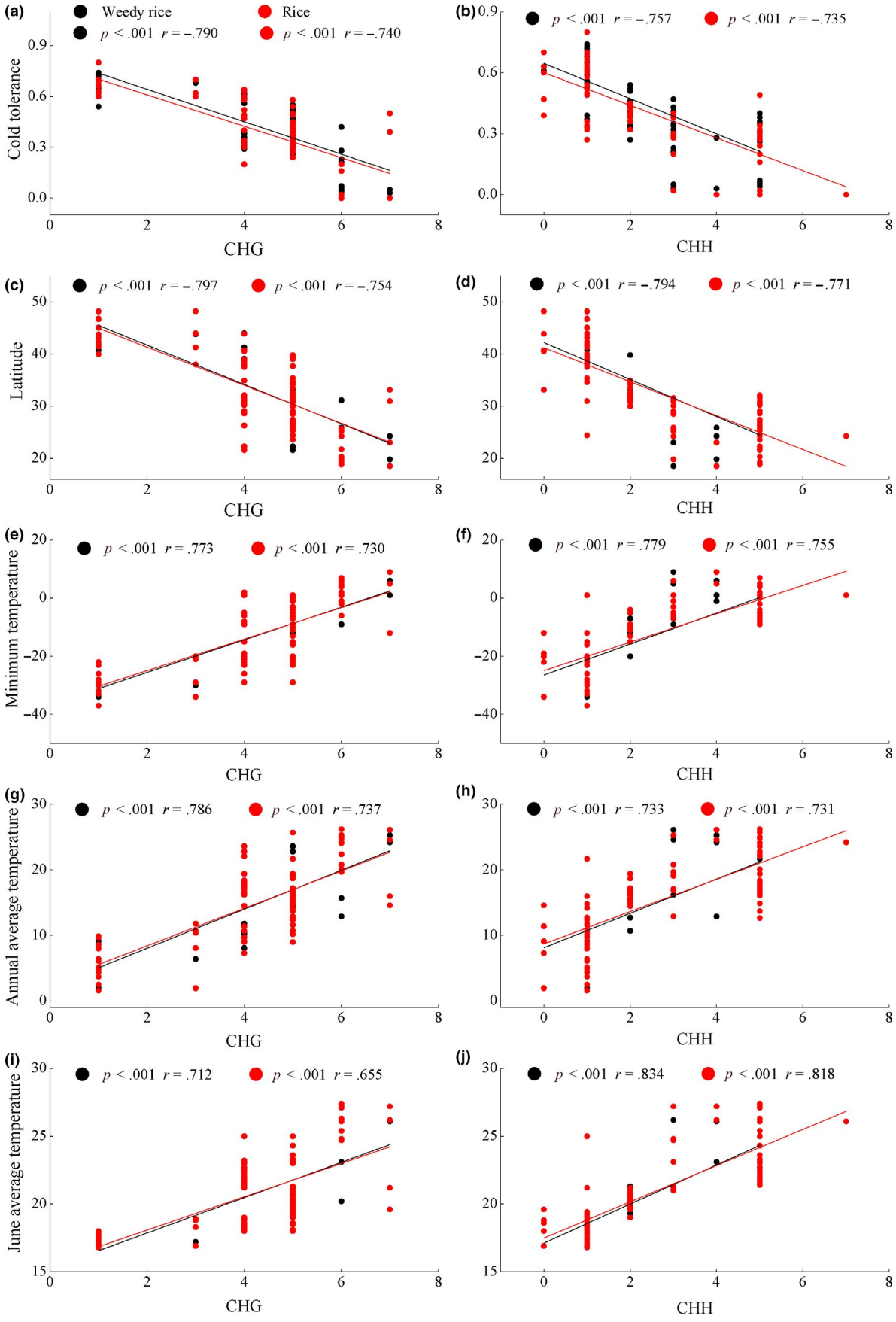

FIGURE 7 Correlation analysis of the CHG and CHH methylation levels of the *OsICE1* promoter and the cold tolerance and geographical information [Colour figure can be viewed at wileyonlinelibrary.com]

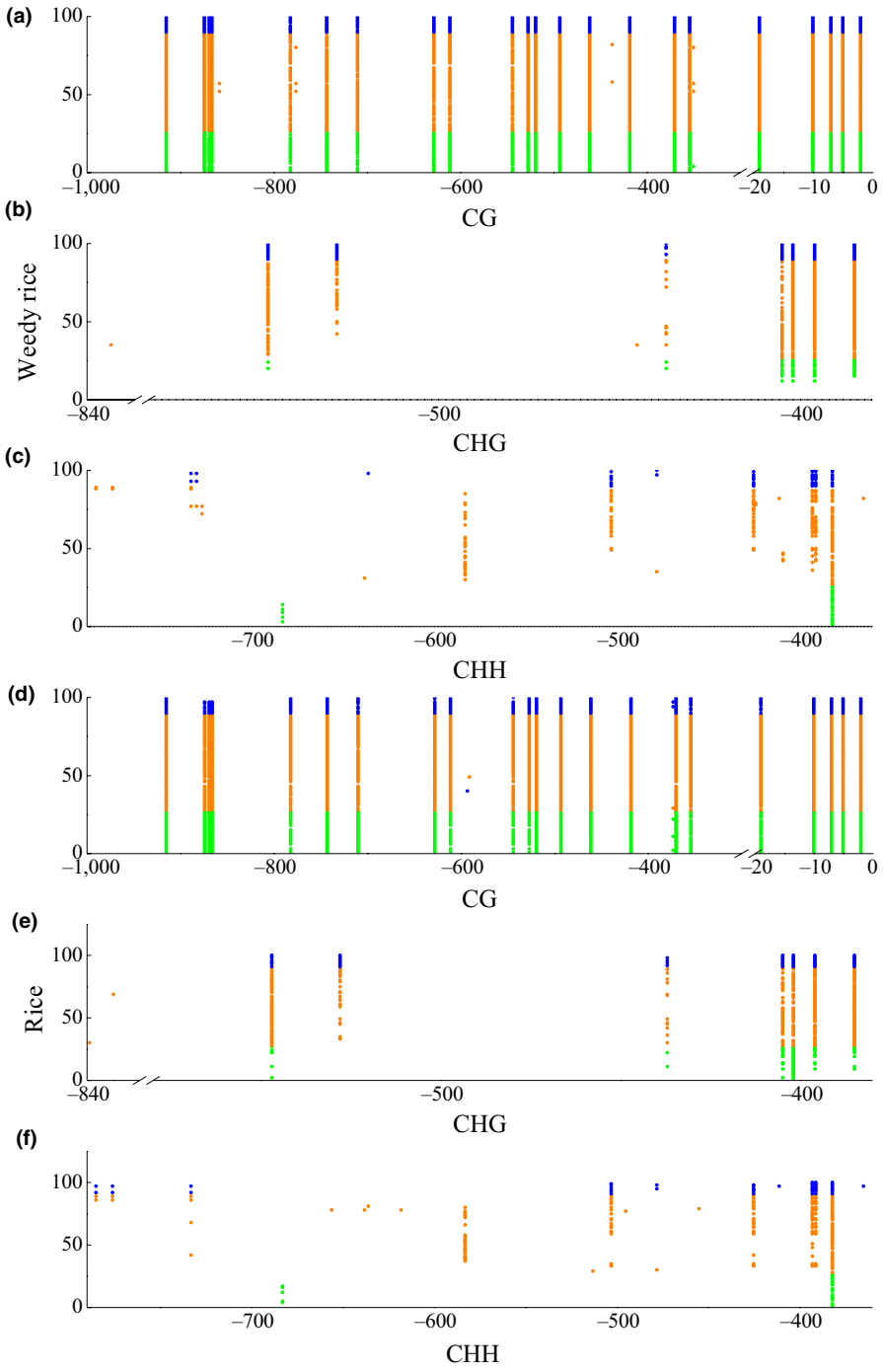

FIGURE 8 Conservation of the *OsICE1* promoter methylation sites. The *x*-axis represents the position of the promoter sequence, numbered from −1 upstream of ATG. The *y*-axis represents the number of weedy rice and rice populations. Green dot: high-latitude populations; orange dot: mid-latitude populations; blue dot: low-latitude populations [Colour figure can be viewed at wileyonlinelibrary.com]

The cold tolerance of weedy rice and rice populations was significantly correlated with *CBF* pathway gene expression levels, indicating that the *CBF* pathway plays important roles in weedy rice and rice cold tolerance regulation. However, the differential expression of *CBF* pathway genes among the different weedy rice and rice populations needs to be further elucidated. Our previous study in invasive crofton weed showed that only *ICE1* coding region had varying methylation levels among the different geographical populations. For that species, methylation of the *ICE1* coding region, including CG, CHG and CHH sites, was negatively correlated with cold tolerance, which may be responsible for its rapid expansion in China (Xie et al., 2015). Studies in other species have shown that the DNA methylation of promoters negatively regulates gene expression (Smith & Meissner, 2013; Zhang et al., 2006); in contrast, CG methylation of the gene body is significantly associated with *Arabidopsis* habitat temperatures (Dubin et al., 2015; Kawakatsu et al., 2016). In our study, the CG methylation level of the *OsICE1* promoter was not correlated with cold tolerance. CHG and CHH methylation of the *OsICE1* promoter was negatively correlated with *CBF* pathway gene expression, which may contribute to the cold tolerance differentiation in weedy rice and rice. These findings indicate that DNA methylation of the *ICE1* coding regions and promoters may play different roles in different plant species.

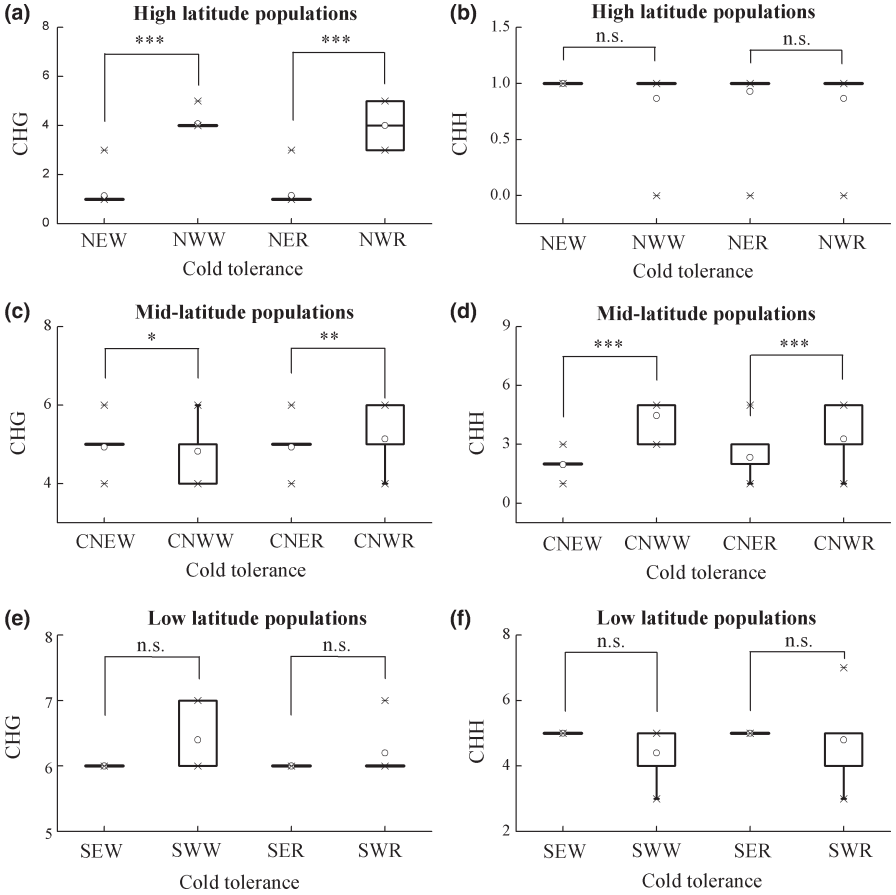

FIGURE 9 Comparison of CHG and CHH methylation levels of the *OsICE1* promoters of weedy rice and rice at different latitudes. CNER, central northeast rice populations; CNEW, central northeast weedy rice populations; CNWR, central northwest rice populations; CNWW, central northwest weedy rice populations; NER, northeast rice populations; NEW, northeast weedy rice populations; NWR, northwest rice populations; NWW, northwest weedy rice populations; SER, southeast rice populations; SEW, southeast weedy rice populations; SWR, southwest rice populations; SWW, southwest weedy rice populations. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

Besides broad geographical patterns of climatic adaptation, there was also cold tolerance differentiation among weedy rice and rice populations at similar latitudes within the high- and mid-latitude regions. The cold tolerance differentiation among populations at high latitudes was correlated with the CHG methylation of the *OsICE1* promoter, while for mid-latitude populations, cold tolerance was correlated with both CHG and CHH methylation levels. It has been reported that the methylation of CG sites is affected by the sample collecting site temperatures in *A. thaliana* (Dubin et al., 2015). However, our findings indicate that CHG and CHH methylation of the *OsICE1* promoter could produce different responses to low temperatures. In moderately low-temperature regions (June average temperatures of 19 ~ 25°C, annual average temperatures of 10.7 ~ 25.7°C, minimum temperatures of −22 ~ 2°C), plants tend to have low CHG and CHH methylation levels. In colder regions (June average temperatures of 16.8 ~ 18.9°C, annual average temperatures of 1.6 ~ 11.8°C, minimum temperatures of −37 ~ 19°C), plants have even lower CHG methylation levels. These findings point to an epigenetic mechanism of the spread of weedy rice concomitant with rice cultivation into northern climates and provide a clue for understanding the morphological and physiological similarity between rice and weedy rice (Delouche et al., 2007; Qiu et al., 2017; Ziska et al., 2015).

The CG methylation sites on the *OsICE1* promoter were relatively stable among the different weedy rice and rice populations, while the CHH methylation sites exhibited the most diversity. The relationship between the cold tolerance differentiation and methylation of the *OsICE1* promoter suggests that the CG methylation of weedy rice and rice cultivars may not play a role in responding to cold temperature and are stable under various climates. The CG methylation did not differ significantly under different temperature conditions and was not correlated with temperature. The CHH methylation differed significantly under various temperatures, leading to the primary adaptation to low temperatures and widespread cultivation areas of weedy rice and rice. The CHG methylation differed in only lower environmental temperatures at high latitudes and had lower methylation diversity than CHH, as it did not differ under primary low-temperature stress. Previous studies have shown that CHH methylation in the gene body is significantly associated with *Arabidopsis* habitat temperatures (Dubin et al., 2015; Kawakatsu et al., 2016), and our study suggested that CHH and CHG methylation of the *OsICE1* promoter is also associated with response to low temperatures. However, additional studies will be required to establish a definitive causal relationship between the observed *OsICE1* promoter methylation patterns and cold tolerance.

The above results reveal for the first time that the convergence of cold tolerance in rice varieties and co-occurring weedy rice populations is correlated with similar methylation levels of their *OsICE1* promoter regions. While the data from this study do not provide evidence on the mechanism by which this adaptive convergence has occurred, we suggest that there are three possibilities. Numerous genetic studies, including recent genome resequencing and population genomic analyses

(Li et al., 2017; Qiu et al., 2017), indicate weedy rice strains worldwide have evolved repeatedly through a process of de-domestication (feralization) from cultivated rice (Wedger & Olsen, 2018). Given that cultivated rice underwent strong selection for local climatic adaptation as rice farming spread into temperate regions, the ability of weedy rice to rapidly adapt across this same range of climates could be a legacy of earlier selection that occurred in its domesticated ancestors. Thus, standing variation in weedy rice populations could provide a reservoir of adaptive variation upon which selection has acted. A second possibility is that, provided sufficiently high mutation rates, adaptive mutations could arise de novo within the weed populations that confer the cold tolerance (and associated changes in CBF pathway gene expression and *OsICE1* promoter methylation). The third possibility is local weed populations have acquired cold tolerance through hybridization with local crop varieties (e.g., Zhang et al., 2015, 2018) and adaptive introgression of cold tolerance. Although both cultivated rice and weedy rice are predominantly self-fertilizing, outcrossing and hybridization does occur (Chen, Dong, Song, Suh, & Lu, 2004; Cui, Dai, Qiang, & Song, 2013; Serrat et al., 2013; Sun, Dai, Cui, Qiang, & Song, 2015; Yao, Jikun, Sheng, Luo, & Song, 2015; Zhang et al., 2015, 2018; Zhang, Linscombe, & Oard, 2003; Zuo, Zhang, Song, Dai, & Qiang, 2011), and this process has been proposed to account for adaptive morphological convergence in populations of weedy rice in the southern United States (Langevin et al., 1990). This mechanism may be less likely, however, as rice cultivars undergo high turnover through much of the rice-growing region of China; as such, the weedy rice samples we collected are unlikely to have acquired introgressed traits from the cultivars we sampled simultaneously. The underlying molecular mechanism of methylation-mediated gene regulation also remains to be elucidated.

The observed correlation between cold tolerance and methylation of the *OsICE1* promoter has important potential applicability for the breeding of cold-tolerant rice varieties. The methylation level of the *OsICE1* promoter can be used as a marker for cold tolerance screening, as it negatively correlates with rice cold tolerance. This method could improve the efficiency and accuracy of crop breeding. By combining methylation-sensitive restriction enzyme and molecular marker technology, we can quickly and efficiently determine the *OsICE1* promoter methylation statuses of a wide range of living plants and screen for cold-tolerant rice cultivars.

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AUTHOR CONTRIBUTIONS

S.Q. designed the study. Y.H., X.L. and H.X. performed the experiments. S.Q. W.D. and X.S. collected the materials. H.X., Y.H., X.L. and S.Q. performed the data analysis. H.X., Y.H. and S.Q. wrote the manuscript. K.M.O. commented on and revised the manuscript.

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DATA AVAILABILITY STATEMENT

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained upon request to the corresponding author. The data can also be obtained from Dryad (https://doi.org/10.5061/dryad.2ch4ns2).

REFERENCES

Agarwal, P. K., Agarwal, P., Reddy, M. K., & Sopory, S. K. (2006). Role of *DREB* transcription factors in abiotic and biotic stress tolerance in plants. *Plant Cell Reports*, *25*(12), 1263–1274. https://doi.org/10.1007/s00299-006-0204-8

Andaya, V. C., & Mackill, D. J. (2003). Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. *Journal of Experimental Botany*, *54*(392), 2579–2585.

Andaya, V. C., & Tai, T. H. (2006). Fine mapping of the qCTS12 locus, a major QTL for seedling cold tolerance in rice. *Theoretical & Applied Genetics*, *113*(3), 467–475.

Bevilacqua, C. B., Basu, S., Pereira, A., Tseng, T.-M., Zimmer, P. D., & Burgos, N. R. (2015). Analysis of stress-responsive gene expression in cultivated and weedy rice differing in cold stress tolerance. *PLoS ONE*, *10*(7), e0132100. https://doi.org/10.1371/journal.pone.0132100

Boyer, J. S. (1982). Plant productivity and environment. *Science*, *218*(4571), 443–448. https://doi.org/10.1126/science.218.4571.443

Chen, L. J., Dong, S. L., Song, Z. P., Suh, H. S., & Lu, B. R. (2004). Gene flow from cultivated rice (*Oryza sativa*) to its weedy and wild relatives. *Annals of Botany*, *93*(1), 67–73. https://doi.org/10.1093/aob/mch006

Chinnusamy, V., & Zhu, J.-K. (2009). Epigenetic regulation of stress responses in plants. *Current Opinion in Plant Biology*, *12*(2), 133–139. https://doi.org/10.1016/j.pbi.2008.12.006

Cokus, S. J., Feng, S., Zhang, X., Chen, Z., Merriman, B., Haudenschild, C. D., … Jacobsen, S. E. (2008). Shotgun bisulfite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature*, *452*(7184), 215–219.

Cruz Damilach, R. P., Kothefederizzi, S. C., & Carlos, L. (2006). Inheritance of rice cold tolerance at the germination stage. *Genetics & Molecular Biology*, *29*(2), 847–853.

Cui, R. R., Dai, W. M., Qiang, S., & Song, X. L. (2013). Gene flow from transgenic glufosinate-resistant rice II you 86B and its restorer line 86B to weedy rice. *Jiangsu Journal of Agricultural Sciences*, *29*(4), 708–714.

Dai, L.-F., Chen, Y.-L., Luo, X.-D., Wen, X.-F., Cui, F.-L., Zhang, F.-T., … Xie, J.-K. (2015). Level and pattern of DNA methylation changes in rice cold tolerance introgression lines derived from *Oryza rufipogon* Griff. *Euphytica*, *205*(1), 73–83. https://doi.org/10.1007/s10681-015-1389-0

Delouche, J. C., Burgos, N. R., Gealy, D. R., Labrada, R. (2007). *Weedy rices: Origin, biology, ecology and control. FAO Plant Production &*

Protection Paper. Rome, Italy: Food and Agriculture Organization of the United Nations.

Dubin, M. J., Zhang, P., Meng, D., Remigereau, M.-S., Osborne, E. J., Paolo Casale, F., … Nordborg, M. (2015). DNA methylation in *Arabidopsis* has a genetic basis and shows evidence of local adaptation. *eLife*, *4*, e05255. https://doi.org/10.7554/eLife.05255

Dubouzet, J. G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E. G., Miura, S., … Yamaguchi-Shinozaki, K. (2003). *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *Plant Journal for Cell & Molecular Biology*, *33*(4), 751–763.

Ehlert, B., & Hincha, D. K. (2008). Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in *Arabidopsis* leaves. *Plant Methods*, *4*(1), 1–7. https://doi.org/10.1186/1746-4811-4-12

Ellstrand, N. C., & Schierenbeck, K. A. (2006). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America*, *148*(1–2), 35–46. https://doi.org/10.1007/s10681-006-5939-3

Fujino, K., Sekiguchi, H., Sato, T., Kiuchi, H., Nonoue, Y., Takeuchi, Y., … Yano, M. (2004). Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). *Theoretical & Applied Genetics*, *108*(5), 794–799.

Guo Zf, O. U., Sy, W. L., & Zhong, Q. (2006). Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. *Plant Physiology and Biochemistry*, *44*(11–12), 828–836.

Hahn, M., & Walbot, V. (1989). Effects of cold-treatment on protein synthesis and mRNA levels in rice leaves. *Plant Physiology*, *91*(3), 930–938. https://doi.org/10.1104/pp.91.3.930

Han, Y. H., Li, X. Y., Xie, H. J., Dai, W. M., & Qiang, S. (2017). Adaptability of rice (*Oryza sativa*) varieties to cold tolerance associated with methylation variation of *ICE1* gene. *Journal of Agricultural Biotechnology*, *25*(9), 1381–1390.

He, Q., Kim, K. W., & Park, Y. J. (2017). Population genomics identifies the origin and signatures of selection of Korean weedy rice. *Plant Biotechnology Journal*, *15*(3), 357–366. https://doi.org/10.1111/pbi.12630

Ishikawa, R., Toki, N., Imai, K., Sato, Y. I., Yamagishi, H., Shimamoto, Y., … Sato, T. (2005). Origin of weedy rice grown in Bhutan and the force of genetic diversity. *Genetic Resources & Crop Evolution*, *52*(4), 395–403.

Ito, Y., Katsura, K., Maruyama, K., Taji, T., Kobayashi, M., Seki, M., … Yamaguchi-Shinozaki, K. (2006). Functional analysis of rice *DREB1/CBF*-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant & Cell Physiology*, *47*(1), 141–153.

Jones, P. A. (2012). Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nature Reviews Genetics*, *13*(7), 484. https://doi.org/10.1038/nrg3230

Kane, N. C., & Baack, E. J. (2007). The origins of weedy rice. *Molecular Ecology*, *16*(21), 4423–4425.

Kawakatsu, T., Huang, S.-S., Jupe, F., Sasaki, E., Schmitz, R. J., Urich, M. A., … Zhou, X. (2016). Epigenomic diversity in a global collection of *Arabidopsis thaliana* accessions. *Cell*, *166*(2), 492–505. https://doi.org/10.1016/j.cell.2016.06.044

Langevin, S. A., Clay, K., & Grace, J. B. (1990). The incidence and effects of hybridization between cultivated rice and its related weed red rice (*Oryza Sativa* L.). *Evolution*, *44*(4), 1000–1008.

Li, H., Qiang, S., & Qian, Y. (2008). Physiological response of different croftonweed (*Eupatorium adenophorum*) populations to low temperature. *Weed Science*, *56*(2), 196–202. https://doi.org/10.1614/WS-07-104.1

Li, L. F., Li, Y. L., Jia, Y., Caicedo, A. L., & Olsen, K. M. (2017). Signatures of adaptation in the weedy rice genome. *Nature Genetics*, *49*(5), 811–816.

Liu, F., Xu, W., Song, Q., Tan, L., Liu, J., Zhu, Z., … Sun, C. (2013). Microarray-assisted fine-mapping of quantitative trait loci for cold tolerance in rice. *Molecular Plant*, *6*(3), 757–767. https://doi.org/10.1093/mp/sss161

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, *25*(4), 402–408.

Lv, Y., Guo, Z., Li, X., Ye, H., Li, X., & Xiong, L. (2015). New insights into the genetic basis of natural chilling and cold shock tolerance in rice by genome-wide association analysis. *Plant Cell & Environment*, *39*(3), 556–570.

Ma, Q., Dai, X., Xu, Y., Guo, J., Liu, Y., Chen, N., … Chong, K. (2009). Enhanced tolerance to chilling stress in *OsMYB3R-2* transgenic rice is mediated by alteration in cell cycle and ectopic expression of stress genes. *Plant Physiology*, *150*(1), 244–256.

Ma, Y., Dai, X., Xu, Y., Luo, W., Zheng, X., Zeng, D., … Chong, K. (2015). *COLD1* confers chilling tolerance in rice. *Cell*, *160*(6), 1209–1221. https://doi.org/10.1016/j.cell.2015.01.046

Mao, D., Yu, L. I., Chen, D., Li, L., Zhu, Y., Xiao, Y., … Chen, C. (2015). Multiple cold resistance loci confer the high cold tolerance adaptation of Dongxiang wild rice (*Oryza rufipogon*) to its high-latitude habitat. *Theoretical and Applied Genetics*, *128*(7), 1359–1371. https://doi.org/10.1007/s00122-015-2511-3

Mitra, R., & Bhatia, C. (2008). Bioenergetic cost of heat tolerance in wheat crop. *Currentence*, *94*, 1049–1053.

Morin, X., Ameglio, T., Ahas, R., Kurz-Besson, C., Lanta, V., Lebourgeois, F., … Chuine, I. (2007). Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. *Tree Physiology*, *27*(6), 817–825. https://doi.org/10.1093/treephys/27.6.817

Mukhopadhyay, A., Vij, S., & Tyagi, A. K. (2004). Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(16), 6309–6314. https://doi.org/10.1073/pnas.0401572101

Nakamura, J., Yuasa, T., Huong, T. T., Harano, K., Tanaka, S., Iwata, T., … Iwaya, M. (2011). Rice homologs of inducer of *CBF* expression (*OsICE*) are involved in cold acclimation. *Plant Tissue Culture Letters*, *28*(3), 303–309. https://doi.org/10.5511/plantbiotechnology.11.0421a

Novillo, F., Alonso, J. M., Ecker, J. R., & Salinas, J. (2004). *CBF2/DREB1C* is a negative regulator of *CBF1/DREB1B* and *CBF3/DREB1A* expression and plays a central role in stress tolerance in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(11), 3985–3990. https://doi.org/10.1073/pnas.0303029101

Oh, C. S., Choi, Y. H., Lee, S. J., Yoon, D. B., Moon, H.-P., & Ahn, S.-N. (2004). Mapping of quantitative trait loci for cold tolerance in weedy rice. *Breeding Science*, *54*(4), 373–380.

Pan, Y. J., Wang, W. S., Zhao, X. Q., Zhu, L., Fu, B., & Li, Z. (2011). DNA methylation alterations of rice in response to cold stress. *Plant Omics*, *4*(7), 364–369.

Qin, Q.-L., Liu, J.-G., Zhang, Z., Peng, R.-H., Xiong, A.-S., Yao, Q.-H., & Chen, J.-M. (2007). Isolation, optimization, and functional analysis of the cDNA encoding transcription factor *OsDREB1B* in *Oryza Sativa* L. *Molecular Breeding*, *19*(4), 329–340. https://doi.org/10.1007/s11032-006-9065-7

Qiu, J., Zhou, Y., Mao, L., Ye, C., Wang, W., Zhang, J., … Lu, Y. (2017). Genomic variation associated with local adaptation of weedy rice during de-domestication. *Nature Communications*, *8*, 15323. https://doi.org/10.1038/ncomms15323

Qiu, J., Zhu, J., Fu, F., Ye, C.-Y., Wang, W., Mao, L., … Fan, L. (2014). Genome re-sequencing suggested a weedy rice origin from domesticated indica-japonica hybridization: A case study from southern China. *Planta*, *240*(6), 1353–1363. https://doi.org/10.1007/s00425-014-2159-2

Reagon, M., Thurber, C. S., Gross, B. L., Olsen, K. M., Jia, Y., & Caicedo, A. L. (2010). Genomic patterns of nucleotide diversity in divergent populations of U.S. weedy rice. *BMC Evolutionary Biology*, *10*(1), 180.

Saijo, Y., Hata, S., Kyozuka, J., Shimamoto, K., & Izui, K. (2000). Overexpression of a single Ca2+-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant Journal*, *23*(3), 319–327.

Sakamoto, A., & Murata, A. N. (1998). Metabolic engineering of rice leading to biosynthesis of glycinebetaine and tolerance to salt and cold. *Plant Molecular Biology*, *38*(6), 1011–1019.

Sanchez, D. H., & Paszkowski, J. (2014). Heat-induced release of epigenetic silencing reveals the concealed role of an imprinted plant gene. *Plos Genetics*, *10*(11), e1004806.

Schreiber, U., Walz, H., & Kolbowski, J. (2003). Propagation of spatial variations of chlorophyll fluorescence parameters in dandelion leaves induced by laser spot heating. *PAM News*, *1*, 1–18.

Serrat, X., Esteban, R., Penas, G., Catala, M. M., Mele, E., & Messeguer, J. (2013). Direct and reverse pollen-mediated gene flow between GM rice and red rice weed. *AoB PLANTS*, *5*(8), plt050. https://doi.org/10.1093/aobpla/plt050

Smith, Z. D., & Meissner, A. (2013). DNA methylation: Roles in mammalian development. *Nature Reviews Genetics*, *14*(3), 204–220.

Song, B. K., Chuah, T. S., Tam, S. M., & Olsen, K. M. (2014). Malaysian weedy rice shows its true stripes: Wild *Oryza* and elite rice cultivars shape agricultural weed evolution in Southeast Asia. *Molecular Ecology*, *23*(20), 5003–5017.

Stoks, R., & De Block, M. (2011). Rapid growth reduces cold resistance: Evidence from latitudinal variation in growth rate, cold resistance and stress proteins. *PLoS ONE*, *6*(2), e16935. https://doi.org/10.1371/journal.pone.0016935

Su, C. F., Wang, Y. C., Hsieh, T. H., Lu, C. A., Tseng, T. H., & Yu, S. M. (2010). A novel MYBS3-dependent pathway confers cold tolerance in rice. *Plant Physiology*, *153*(1), 145–158.

Sun, G., Dai, W., Cui, R., Qiang, S., & Song, X. (2015). Gene flow from glufosinate-resistant transgenic hybrid rice Xiang 125S/Bar68-1 to weedy rice and cultivated rice under different experimental designs. *Euphytica*, *204*(1), 211–227. https://doi.org/10.1007/s10681-015-1370-y

Sun, J., Qian, Q., Ma, D.-R., Xu, Z.-J., Liu, D., Du, H.-B., & Chen, W.-F. (2013). Introgression and selection shaping the genome and adaptive loci of weedy rice in northern China. *New Phytologist*, *197*(1), 290–299. https://doi.org/10.1111/nph.12012

Tao, Z., Kou, Y., Liu, H., Li, X., Xiao, J., & Wang, S. (2011). *OsWRKY45* alleles play different roles in abscisic acid signalling and salt stress tolerance but similar roles in drought and cold tolerance in rice. *Journal of Experimental Botany*, *62*(14), 4863–4874. https://doi.org/10.1093/jxb/err144

Thomashow, M. F. (1999). Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. *Annual Review of Plant Physiology and Plant Molecular Biology*, *50*(1), 571–599. https://doi.org/10.1146/annurev.arplant.50.1.571

Thomashow, M. F. (2010). Molecular basis of plant cold acclimation: Insights gained from studying the *CBF* cold response pathway. *Plant Physiology*, *154*(2), 571–577.

Urano, K., Kurihara, Y., Seki, M., & Shinozaki, K. (2010). 'Omics' analyses of regulatory networks in plant abiotic stress responses. *Current Opinion in Plant Biology*, *13*(2), 132–138.

Wang, G.-J., Miao, W., Wang, J.-Y., Ma, D.-R., Li, J.-Q., & Chen, W.-F. (2013). Effects of exogenous abscisic acid on antioxidant system in weedy and cultivated rice with different chilling sensitivity under chilling stress. *Journal of Agronomy & Crop Science*, *199*(3), 200–208. https://doi.org/10.1111/jac.12004

Wang, H., Beyene, G., Zhai, J., Feng, S., Fahlgren, N., Taylor, N. J., … Ausin, I. (2015). CG gene body DNA methylation changes and evolution of duplicated genes in cassava. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(44), 13729–13734. https://doi.org/10.1073/pnas.1519067112.

Wang, P., Zhang, Z., Song, X., Chen, Y. I., Wei, X., Shi, P., & Tao, F. (2014). Temperature variations and rice yields in China: Historical contributions and future trends. *Climatic Change*, *124*(4), 777–789. https://doi.org/10.1007/s10584-014-1136-x

Wedger, M. J., & Olsen, K. M. (2018). Evolving insights on weedy rice. *Ecological Genetics and Genomics*, *7*, 23–26.

Xia, H. B., Xia, H., Ellstrand, N. C., Yang, C., & Lu, B. R. (2011). Rapid evolutionary divergence and ecotypic diversification of germination behavior in weedy rice populations. *New Phytologist*, *191*(4), 1119–1127. https://doi.org/10.1111/j.1469-8137.2011.03766.x

Xie, H. J., Li, H., Liu, D., Dai, W. M., He, J. Y., Lin, S., … Qiang, S. (2015). *ICE1* demethylation drives the range expansion of a plant invader through cold tolerance divergence. *Molecular Ecology*, *24*(4), 835–850.

Yang, A., Dai, X., & Zhang, W. H. (2012). A R2R3-type MYB gene, *OsMYB2*, is involved in salt, cold, and dehydration tolerance in rice. *Journal of Experimental Botany*, *63*(7), 2541–2556. https://doi.org/10.1093/jxb/err431

Yao, H., Jikun, L. I., Sheng, Q., Luo, T., & Song, X. (2015). Gene flow from transgenic rice T1c–19 with stacked cry1C*/bar genes to weedy and cultivated rice species. *Chinese Journal of Applied & Environmental Biology*, *21*(6), 1112–1119.

Zhang, J., Kang, Y. E., Valverde, B. E., Dai, W., Song, X., & Qiang, S. (2018). Feral rice from introgression of weedy rice genes into transgenic herbicide-resistant hybrid-rice progeny. *Journal of Experimental Botany*, *69*(16), 3855–3865. https://doi.org/10.1093/jxb/ery210

Zhang, J., Lu, Z., Dai, W., Song, X., Peng, Y., Valverde, B. E., & Qiang, S. (2015). Cytoplasmic-genetic male sterility gene provides direct evidence for some hybrid rice recently evolving into weedy rice. *Scientific Reports*, *5*, 10591. https://doi.org/10.1038/srep10591

Zhang, N., Linscombe, S., & Oard, J. (2003). Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice. *Euphytica*, *130*(1), 35–45.

Zhang, Q., Chen, Q., Wang, S., Hong, Y., & Wang, Z. (2014). Rice and cold stress: Methods for its evaluation and summary of cold tolerance-related quantitative trait loci. *Rice*, *7*(1), 24. https://doi.org/10.1186/s12284-014-0024-3

Zhang, Q., Jiang, N., & Wang, G. L. (2013). Advances in understanding cold sensing and the cold-responsive network in rice. *Advances in Crop Science and Technology*, *1*(1), 1.

Zhang, X., Yazaki, J., Sundaresan, A., Cokus, S., Chan, S.-L., Chen, H., … Ecker, J. R. (2006). Genome-wide high-resolution mapping and functional analysis of DNA methylation in *arabidopsis*. *Cell*, *126*(6), 1189–1201. https://doi.org/10.1016/j.cell.2006.08.003

Zilberman, D., Gehring, M., Tran, R. K., Ballinger, T., & Henikoff, S. (2007). Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nature Genetics*, *39*(1), 61–69. https://doi.org/10.1038/ng1929

Ziska, L. H., Gealy, D. R., Burgos, N., Caicedo, A. L., Gressel, J., Lawton-Rauh, A. L., … Merotto, A., Jr. (2015). Weedy (red) rice: An emerging constraint to global rice production. *Advances in Agronomy*, *129*, 181–228.

Zuo, J., Zhang, L., Song, X., Dai, W., & Qiang, S. (2011). Innate factors causing differences in gene flow frequency from transgenic rice to different weedy rice biotypes. *Pest Management Science*, *67*(6), 677–690. https://doi.org/10.1002/ps.2108

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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